FEATURE

Balancing Silence:

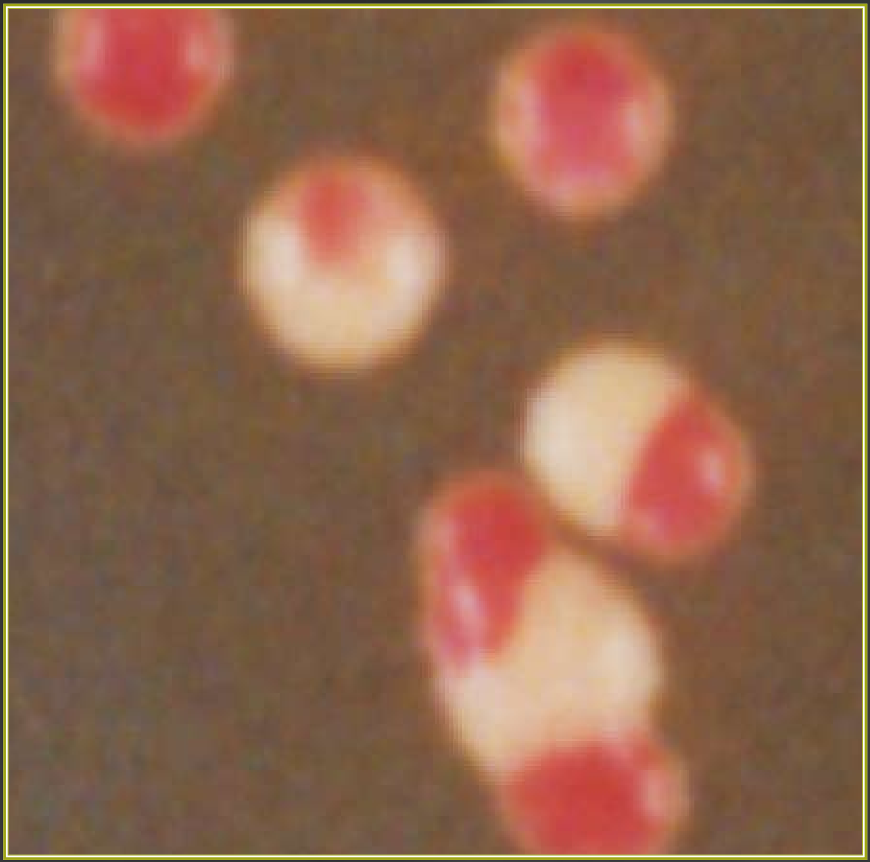
How a Cell's Fate Is Determined

The fate of a cell is determined by more than the string of A's, T's, G's, and C's that make up its DNA sequence. Epigenetic regulation of gene expression—governed by the way in which DNA and proteins are packed and interact in the tiny nucleus of a cell—is the reason why two cells with identical DNA can have very different characteristics. For example, one might be a neuron producing electrical signals and the other a pancreatic islet cell producing insulin. **Shiv Grewal, Ph.D.**, *Head of the Chromosome Biology section in CCR's Laboratory of Biochemistry and Molecular Biology, has studied this question of phenotypic determination in the fission yeast, Schizosaccharomyces pombe, for more than a decade. The mechanisms that he and his team have uncovered appear fundamental to maintaining genomic stability and function, from yeast to man.*

The Mating Habits of Yeast

Among the model organisms biologists use to study genetic mechanisms, the fission yeast *Schizosaccharomyces pombe* has several advantages. A single-celled eukaryotic organism with a small genome that divides rapidly and is easily grown in a laboratory Petri dish, *pombe* nonetheless turns out to share many conserved cellular mechanisms with higher eukaryotes, including man.

For such simple creatures, fission yeast have a complicated sex life that is defined by a single location on their genome—the mating type locus *(mat)*. *Mat* encodes three genes, but two of these genes are silenced, and only one is actually expressed in a single organism, thereby defining it as being either an M- or P- mating cell type. Feeding happily in a nutritionally rich environment, fission yeast do not reveal their mating type; it is only when starved that they define their orientation and partner with a cell of the other type to reproduce.



(*Image:* S.Grewal, CCR)

Fission yeast colonies composed of cells with identical genomes. Red colonies indicate silencing of a gene caused by the spreading of heterochromatin complexes.

Fission yeast can also reproduce asexually, so that a single cell divides to form a colony of clones. What has fascinated cell biologists for decades, however, is the fact that a single clone can give rise to both mating cell types. What genetic mechanisms could account for the mating type switch of just one of two daughter cells? Although this might seem like a somewhat esoteric question in yeast biology, it addresses the most basic notion of how two cells with identical genomes can, in fact, be different. In *pombe*, this vast question comes down to the more experimentally tractable one of how gene silencing at the *mat* locus is controlled—a question Grewal first set out to answer as a Postdoctoral Fellow in the laboratory of Amar Klar, Ph.D. (now Head of the Developmental Genetics Section in CCR's Gene Regulation and Chromosome Biology Laboratory), and has continued working on to this day.

(Photo: R. Baer)

Shiv Grewal, Ph.D., examines colonies of fission yeast.

> [...it addresses the most basic notion of how two cells with identical genomes can, in fact, be different.]

Good Chromatin and the Rest

Packaging a double-stranded DNA helix measuring a meter or more in length into the nucleus of a cell measuring less than a millimeter is no small feat of molecular engineering. The genome is highly compacted, with DNA wound around protein complexes called histones to form repeating structures called nucleosomes, which are themselves folded into even more complex structures that eventually form what microscopists see as chromatin material (chromosomes) in the nucleus. Modifying the histone protein complexes by enzymes is an important means of regulating gene expression (see "Histone Modification and Cancer").

Long before Watson and Crick deduced the double helical structure of DNA, cell biologists described the genetic material within the cell nucleus as occurring in two forms—heterochromatin and euchromatin—on the basis of their staining patterns under a light microscope. Heterochromatin is a highly condensed form of chromatin in which gene expression is largely silenced, whereas euchromatin is much more loosely configured and enriched with expressed genes. Heterochromatin typically contains DNA with long repeating elements that do not encode genes. Instead, it comprises structurally distinct and important regions of the chromosome such as telomeres (the ends of the chromosomes that need to be protected from enzymes) and centromeres (where the two halves of a chromosome are joined in the middle and where the machinery that segregates chromosomes during cell division attaches).

"For a long time, heterochromatin was looked upon as part of the genome that is silenced as inert static structures," recalled Grewal. "But we know now they are highly dynamic structures that change in response to the cell cycle, developmental and environmental conditions, and stresses. Heterochromatin plays a very important role in a number of cellular processes, including developmental choices." Grewal has been an integral part in creating this new view of heterochromatin.

The Role of RNAi

It turns out that gene silencing at the mating-type locus and centromeres in fission yeast is controlled through heterochromatin. Early on in his work on mating type switching, Grewal set out to sequence the whole region. "While I was sequencing, I found a repeat element in the middle and thought I had accidentally cloned centromeric repeats," he recalled. Instead, he had discovered that the mating type region also contained these repeats and, furthermore, that knocking out these repeats abolished silencing across the region.

After his postdoctoral fellowship, Grewal started his own laboratory in Cold Spring Harbor in 1998. Searching for new genetic mutations that would alter mating type switching in fission yeast to provide new insights into the mechanism, his laboratory had knocked out the gene *ago* (*argonaute*) because it had recently been shown to affect the similar phenomenon of asymmetric cell division in germ cells. "For six months, we had no phenotype," reported Grewal. "But then I noticed that the cells with *ago* had chromosome segregation problems. These were easy for me to recognize because other factors I worked with as a postdoc, which affected heterochromatic silencing at centromeres, also had chromosome segregation problems." Therefore, he reasoned, *ago* may also be involved in the regulation of heterochromatin.

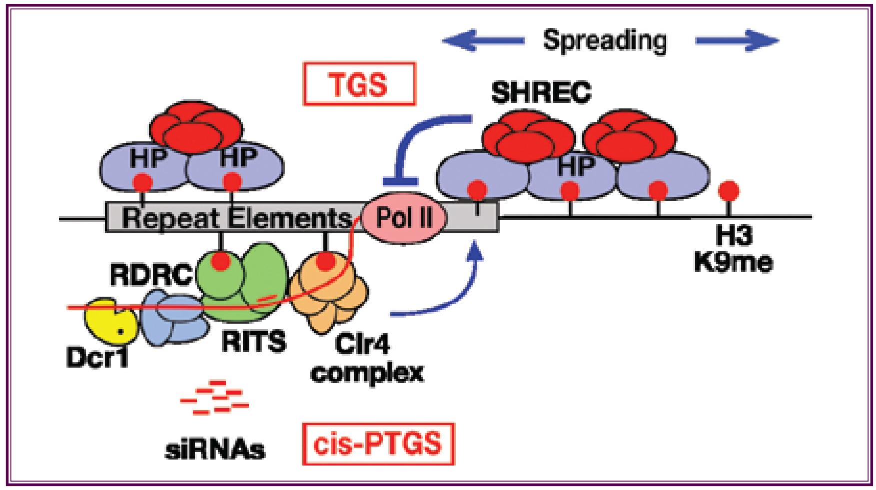
"A lot of things come together in science sometimes," remarked Grewal. Around this time, Craig Mello, Ph.D., published his finding that in worms argonaute was part of the newly described process of RNA interference (RNAi).

RNAi acts by the production of very small pieces of RNA from double-stranded RNA generated at specialized repeating DNA elements. RNAi acts through multiple pathways to regulate gene transcription and translation. (Mello shared the Nobel Prize with his colleague Andrew Fire, Ph.D., for the discovery of this process in 2006.) Meanwhile, Grewal and his Postdoctoral Fellows Junichi Nakayama, Ph.D., and Ken-ichi Noma, Ph.D., were getting ready to publish two papers in *Science* demonstrating that a specific methylation of the histones (designated H3K9) was important for gene silencing at the *mat* locus through the recruitment of so-called chromodomain proteins. The next step was to show that RNAi was necessary both for H3K9 methylation and for gene silencing. Over the next few years, working with his student Ira Hall and Postdoctoral Fellow Ken-ichi Noma, Grewal did just that.

"The key thing from all these studies…is a self-reinforcing loop," explained Grewal. H3K9 methylation in heterochromatin provides a landing pad for RNAi machinery, which can in turn act on the repeating DNA sequences in the heterochromatin to further recruit silencing machinery. Silencing can also spread from the region of initiation across long stretches of DNA. In fact, studies by Postdoctoral Fellows Takatomi Yamada, Ph.D., Tomoyasu Sugiyama, Ph.D., Songtao Jia, Ph.D., and Tamas Fischer, Ph.D., have led to an important realization that heterochromatin serves as a versatile recruiting platform for factors involved in many cellular processes, including for proteins involved in cell-type switching and proper segregation of chromosomes during cell division.

Blurring the Distinction

"Heterochromatin is not a static, inert structure," repeated Grewal. "The [chromodomain] proteins not only recruit silencing proteins but also recruit destabilizers to promote transcription… it's a balance that determines the state." In particular, work performed by Postdoctoral Fellow Martin Zofall, Ph.D., showed that a single chromodomain protein (called Swi6) recruits not only silencing factors but also anti-silencing factors that facilitate transcription of heterochromatic repeats. Moreover, a paper published in



(Image: S. Grewal, CCR)

Formation of heterochromatin in fission yeast involves coordinated functions of multiple proteins. A self-reinforcing loop couples the generation of short interfering RNAs (siRNAs*, indicated as red line segments*) from DNA repeat elements with the methylation of histones (H3K9me) via the Clr4 complex. The chromodomain proteins (HP), which bind to methylated histones, recruit gene silencing factors (SHREC). Spreading of heterochromatin allows its associated factors to exert influence over extended chromosomal domains.

> "Heterochromatin is not a static, inert structure."

Nature in February 2008 describes the work led by Grewal's Postdoctoral Fellows Ee Sin Chen, Ph.D., and Ke Zhang, Ph.D., to demonstrate that heterochromatin actually changes during the process of cell division. As cells enter the S or "synthesis" phase, there is a 20-minute window in which DNA at the centromere is actually transcribed, due to a temporary destabilization of the heterochromatin and an active recruitment of the transcriptional machinery involving RNAi complexes. Transcription appears to be necessary in this phase for promoting the proper recruitment of factors controlling heterochromatin silencing during cell division.

In fact, the difference between heterochromatin and euchromatin may actually be a matter of degree—similar mechanisms appear to operate in both, but to different degrees. Recently, Grewal's lab has been looking at "the rest of the genome" and not just the heterochromatin regions of the telomeres, centromeres, and the *mat* locus. "It is argued that RNAi controls repetitive parts of the genome," explained Grewal. However, repeating DNA elements are not restricted to the classic heterochromatic regions. The eukaryotic genome is littered with smaller repeating elements like retrotransposons that were integrated from the genetic material encoded by invading viruses over the course of countless generations. Grewal and his team wanted to know what repressed the expression of these elements.

Postdoctoral Fellow Hugh Cam, Ph.D., led another study that was also published in *Nature* in January 2008 (see "Transposon, Regulate Thyself" in Vol. 2, No. 2 of *CCR connections*). Initially, the team found that a set of proteins in *pombe* related to the human CENP-B protein involved in centromere formation was bound to retrotransposons scattered throughout the genome. Eliminating the genes that code for these CENP-B homologues released these genes from their transcriptional repression. Most interestingly, the CENP-B homologues recruited much of the same machinery to silence gene expression as is found in heterochromatin.

"The cell has a toolkit of repressors [of gene transcription]," concluded Grewal. Silencing of a large chromosomal domain requires a repeat element and RNAi to recruit the repressor complexes associated with heterochromatin and cause it to spread. Now, the same repressors are also silencing other repeat elements in the genome but by a mechanism involving CENP-B homologues that does not cause spreading

(Photo: R. Baer)

Shiv Grewal, Ph.D. *(second from the right in front)*, and current members of his laboratory study the mechanisms of epigenetic regulation.

repression. "Again and again, effectors that are on heterochromatin are there on euchromatin, but their targeting differs. If you think in biochemical terms, the differences between heterochromatin and euchromatin are disappearing. It's really a dynamic balance—in heterochromatin regions, the balance has shifted to more repressors; in euchromatin regions, the balance favors transcription."

Finding New Challenges

"I knew I wanted to study how chromatin and its complexes modify gene expression with a particular angle to epigenetic regulation, but I didn't really expect that in 10 years time, we'd know the rough outline of the major pathways," Grewal said. "The challenge for us is to keep on finding more interesting issues to explore, and so far we haven't run out."

Grewal looks forward to the day when he will be able to study chromatin states at the level of a single cell; since most of their work is based on material extracted from populations of cells, information about individual variability is lost. But he knows that it will take some time to get there.

He also believes that new breakthroughs will come from what people currently describe as "unwanted transcription." DNA is meant to be read by transcriptional machinery in one direction only to produce a functional gene. Two papers have recently appeared, however, reporting that most of the fission yeast genome is transcribed in both directions. "As you look at it more closely, it may have biological implications," suggested Grewal.

"We are fortunate to be in a lab where we share a lot of interests with the people around us," Grewal said, pointing to the CCR's Center of Excellence in Chromosome Biology. He used to feel that his focus should remain exclusively on a deep understanding of the basic mechanisms of epigenetic regulation but senses that the time may soon come to explore further afield. "Being at NCI will provide us with the opportunity to work with other colleagues to apply some of our knowledge. Heterochromatin is at the center of genome stability. What happens in different cancer cell types to heterochromatin structures? Are there ways we can re-engineer these broken pathways?" Grewal notes that histone deacetylase inhibitors are already used in the treatment of some cancers (see "Histone Modification and Cancer").

"I've been very grateful for the support of CCR's directors," concluded Grewal. "Sometimes it's hard to point out the relevance of these things in the context of cancer, but if you look deeply, you see the implications which are usually borne out with time."

To read more about Dr. Grewal's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=grewal.

Histone Modification and Cancer

Histone proteins interact with DNA to regulate the structure of chromatin material and the access of machinery to transcribe or silence genes. Enzymes add small molecule groups to these proteins, and these modifications influence this regulation. The most common modifications are the addition of an acetyl group (acetylation) or a methyl group (methylation) to the histone protein. Altered function in both of these processes has been linked to cancer.

The enzyme histone deacetylase (HDAC) removes acetyl groups. In his early work on heterochromatin in fission yeast as a Postdoctoral Fellow, Shiv Grewal, Ph.D., actually worked with HDAC mutants before it was known what they were. All he knew at the time was that in these mutants (*clr3* and *clr6*), the normal repression of genes in the *mat* locus and centromeres was disrupted. Since then, he and others have shown that HDACs are fundamental to transcriptional repression.

Increasing evidence indicates that the enzymes that regulate histone acetylation—HDACs and histone acetyl transferases (HATs)—are altered in several cancers. Several HDAC inhibitors are currently in various phases of clinical testing for effectiveness as anti-cancer agents (see "Radiating Change," page 28). Although the mechanism of action is not certain, HDAC inhibitors may relieve the repression of tumor suppressor genes.